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Kinetics of First Spermatogenesis in Young Toads of *Xenopus laevis*

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ABSTRACT—The kinetic profile of first spermatogenesis was examined at 22±1°C in young toads of *Xenopus laevis*. The most progressed spermatogenic stage was primary spermatogonia in newly metamorphosed toads. Secondary spermatogonia were observed 3 days after metamorphosis. First leptotene and pachytene spermatocytes appeared 17 and 20 days after metamorphosis, respectively. Then first diplotene spermatocytes, meiotic division and round spermatids were observed 42 days, and first mature spermatozoa 70 days, after metamorphosis. On the basis of these results, the difference between the time required for first spermatogenesis in young toads and cycling spermatogenesis in adult toads is discussed.

INTRODUCTION

The kinetics of spermatogenesis in amphibians has been studied by several investigators, but the materials used in their studies were mostly subadult and adult animals [2, 4, 9, 17]. The formation of spermatozoa in juvenile frogs has been reported as precocious spermatogenesis in several species [5, 7, 22, 23], but these papers simply describe the phenomenon. Recently, we defined the kinetics of cycling spermatogenesis at 22°C in adult toads of *Xenopus laevis* [2]. In the present study, the time after metamorphosis at which the most rapidly progressed spermatogenic cells appeared at 22°C is examined in young toads of this species, and difference between these results and the kinetics of cycling spermatogenesis in adult toads was discussed.

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MATERIALS AND METHODS

Numerous fertilized eggs were obtained from a few pairs of male and female toads *Xenopus laevis* injected with a human chorionic gonadotropin (Gonatropin, Teikoku Zoki Co., Tokyo). The eggs, embryos, and larvae were kept in dechlorinated tap water at 22±1°C. The larvae and young toads were fed on a commercial diet for carp. These details were previously described [3, 8].

Until the time of the first appearance of spermatozoa after metamorphosis, three to four young toads were decapitated every day, and the testes were fixed in Bouin's solution. The testes were then embedded in Paraplast (Sherwood Medical, St. Louis, U.S.A.), and sectioned serially at 4 μm. The sections were stained with Carazzi's hematoxylin and eosin.

RESULTS AND DISCUSSION

In this study, the average body length just after metamorphosis was 15 mm, and was 20 mm at the end of experiment (82 days after metamorphosis). As previously reported [8], seminiferous tubules were clearly observed in young toads just after metamorphosis. At this time (0 day after metamorphosis), the seminiferous tubules were filled with primary spermatogonia. The days on which

Table 1. The most rapidly progressed spermatogenic stage of first spermatogenesis in *Xenopus laevis*

Days after metamorphosis	Stage
0	primary spermatogonium
3–16	secondary spermatogonium
17–19	primary spermatocyte (leptotene)
20–29	zygotene
30–41	pachytene
42–64	diplotene-round spermatid
65–69	elongated spermatid
70–82	spermatozoon

the most rapidly progressed spermatogenic stages were observed are shown in Table 1. The first appearance of spermatocytes and spermatids was observed on 17 days and 42 days after metamorphosis, respectively. Elongated spermatids were seen 65 days after metamorphosis. Seventy days after metamorphosis, the first spermatozoa were observed, though the number is a few (Fig. 1a). When the round spermatids differentiated into the elongated spermatids (from 45 to 70 days after metamorphosis), pycnotic germ cells were frequently observed (Fig. 1b).

From the results shown in Table 1, the duration of each spermatogenic process was calculated. The duration of leptotene, zygotene, and pachytene spermatocytes was 3, 10, and 12 days. The progression from the diplotene spermatocytes to the round spermatids was accomplished within one day. The duration of spermiogenesis was 28 days, whereas the progression of elongated spermatids to spermatozoa was 5 days.

In a previous study, we determined the time required for the progression of cycling spermatogenesis at 22°C in adult *X. laevis* ([2], Kobayashi, unpublished). Although the time required for the progression from elongated spermatids to spermatozoa was similar between young and adult toads (young toads: 5 days, adult toads: 4 days), the present study (Table 1) indicates that the progression from round spermatids to elongated spermatids in young toads was greatly delayed compared with that in adult toads [2] (young toads: 23 days, adult toads: 4 days). In this study, furthermore, during spermiogenesis the degeneration of germ cells, probably round to elongated spermatids, was frequently observed. From these facts, it is suggested that the delay of spermiogenic progression in first spermatogenesis is caused by the failure of nuclear elongation of spermatids. It seems, therefore, that the time required for the progression from round to elongated spermatids in first spermatogenesis is the real time required for the progression from round to elongated spermatids plus the time required for the appearance of round spermatids which could be differentiated into elongated spermatids. Thus, it is calculated that first appearance of round spermatids which

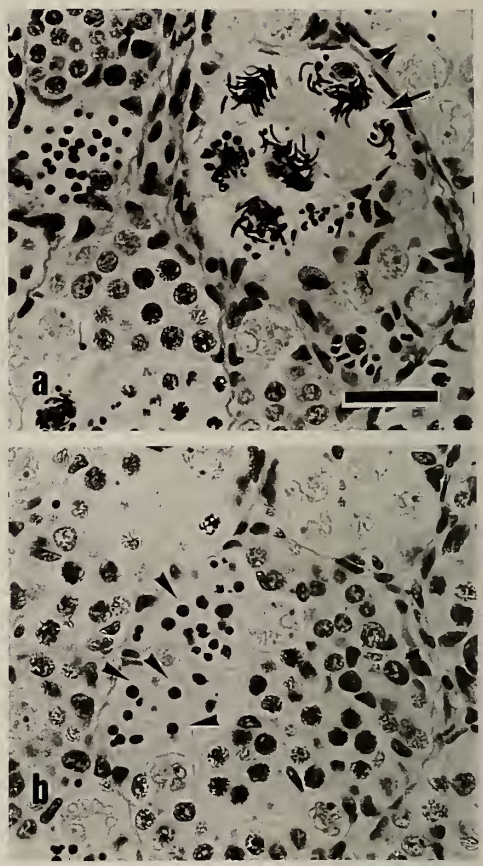

Fig. 1. First spermatogenesis in *X. laevis*. a: First spermatozoa (arrow) are seen 70 days after metamorphosis. b: Pycnotic substances (arrow head) are frequently seen from 45 to 70 days after metamorphosis. Scale bar: 30 μm.

could be differentiated into elongated spermatids is 19 days after the first appearance of round spermatids, if the duration of progression from round to elongated spermatids is similar to that in adult toads as in mice [21]. To define the dynamics in first spermatogenesis, further studies will be necessary.

In *X. laevis* and *Cynops pyrrhogaster,* it is known that in an *in vitro* cell culture system progression from primary spermatocytes to elongated spermatids was accomplished but spermiogenesis failed to be completed [1]. When nuclear elongation proceeds *in vivo,* in *X. laevis,* a few of microtubules contained in spermatids were cast off along with cytoplasm and numerous microtubules in Sertoli cells were situated approximately parallel to the axis of spermatids elongation in the area which immediately surround the spermatids [18]. In *X. laevis* [19], furthermore, progression from premeiotic S spermatogenic cells to mature spermatozoa was accomplished in an *in vitro* organ culture system. Thus, in *X. laevis,* it seems that Sertoli cells play the primary role for the nuclear elongation of spermatids. Therefore, the failure of the progression from round to elongated spermatids may be caused by the lack of functional Sertoli cells and/or abnormality of spermatids, though the functional characterization of Sertoli cells and spermatogenic cells was not clarified in details.

In *Rana esculenta* it was suggested that the completion of spermiogenesis required androgen [15, 16]. Concerning the experiment which dealt with precocious spermatogenesis in *Rana nigromaculata,* we pointed out that androgen was needed for the completion of spermiogenesis [10, 11, 24, 25]. In immature rats, on the other hand, it was reported that an increase of luteinizing hormone binding sites in Leydig cells was induced by prolactin (PRL), though this phenomenon was accompanied by the decrease of PRL-binding sites [12]. On this point, in anurans, it is known that serum PRL levels are high at the climax of metamorphosis [23]. In *X. laevis*, it was reported that gonadotropin- and PRL-producing cells were observed at premetamorphic stages [13, 14]. Furthermore, it was reported that PRL binding sites was present in testes [6]. Although, at present, the information on these pituitary hormonal levels and gonadal steroid levels in young toads of *X. laevis* is few, defined steroid producing cells in the testes were not observed electron microscopically 70 days after metamorphosis (Kobayashi, unpublished). Therefore, it is possible that androgen is involved in spermiogenic process. In this study, however, whether or not the relationship between PRL and androgen production during first spermatogenesis is not known.

In conclusion, the present study indicates in *X. laevis* that the duration of spermatogenesis in first spermatogenesis in young toads is much longer than that of cycling spermatogenesis in adult toads. This delay may be caused by the failure of nuclear elongation of spermatids.

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